from the field testing can be correlated with gene expression during early embryogenesis to further enhance the database of the present invention. This will allow further identification of gene products which whose expression is correlated, either positively or negatively, with commercially valuable tree characteristics.

[0114] It will be clear to those skilled in the art that identification of such gene products can have several uses. Determining the correlation between a desirable phenotype and a genotype would allow for the "pre-selection" of tree embryos for field testing. It would also be useful in evaluating experimental tissue culture conditions for somatic embryogenesis; in other words, the expression level of a gene known to correlate with the development of trees with desirable characteristics could serve as the criterion on which culture media is evaluated, as opposed to assessing the phenotype of fully matured trees. The ability to evaluate culture conditions without having to develop fully mature trees and do field testing would save a great deal of research time and expense. And of course, the knowledge of the correlation between gene expression and desirable tree phenotypes would serve to identify target genes for genetic engineering.

Genetically Engineering Trees and Other Plants

[0115] There are several methods known in the art for the creation of transgenic plants. These include, but are not limited to: electroporation of plant protoplasts, liposome-mediated transformation, polyethylene-glycol-mediated transformation, microinjection of plant cells, and transformation using viruses. Because the invention is especially concerned with the transformation of woody species, the two prevalent methods for transforming forest trees, namely *Agrobacterium*-mediated transfer and

LAW OFFICES
FINNEGAN, HENDERSON, FARABOW, GARRETT, & DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

direct gene transfer by particle bombardment, will be discussed in more detail, though it is understood that the present invention encompasses generation of transgenic plants via standard methods commonly known in the art.

Agrobacterium Mediated Transfer

[0116] *A. tumefaciens* and *A. rhizogenes* are two soil microorganisms that naturally infect a wide variety of plants including dicotyledonous plants, gymnosperms and some monocotyledonous plants. Infection by these organisms results in the growth of crown gall tumors or in hairy root disease, respectively. Each of these organisms carries a large plasmid, the tumor inducing (Ti) plasmid, in the case of *A. tumefaciens* and the root-inducing (Ri) plasmid in the case of *A. rhizogenes*. These plasmids have two critical features, a set of virulence genes and a segment of DNA called T-DNA that is delimited by conserved regions of approximately 25 base pairs known as the left and right borders. During infection, the T-DNA is transferred to the plant cell where it is able to stably integrate in single copy in the plant genome. Transfer of T-DNA requires the function of the virulence genes.

[0117] In its natural state, T-DNA contains genes that mediate progression of disease such as growth hormones or genes controlling root morphogenesis. Using recombinant DNA technology, however, T-DNA may be modified to contain an expression cassette encoding a foreign gene of interest. There are several T-DNA vector systems commonly in use for the transformation of plants. Several of these vector systems are reviewed in Hansen et al., *Current Topics in Microbiology and Immunology* 240: 21-57 (1999) which is hereby incorporated by reference. T-DNA vectors must include the left and right borders. In addition they must either be capable

LAW OFFICES
FINNEGAN, HENDERSON, FARABOW, GARRETT, & DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000

of replication in *Agrobacterium* or be designed so as to recombine with a plasmid that does so. The latter type of vector is known as a co-integrate vector. For transformation to proceed, there must also be a source of virulence (*vir*) genes. The *vir* genes may be on the same plasmid with the T-DNA or more likely supplied by a helper plasmid. For example, binary T-DNA vector systems are comprised of two plasmids, one containing the vir genes and the other containing T-DNA. Some plants known to be recalcitrant to Agrobacterium-mediated transformation may be transformed if additional copies of some or all virulence genes are provided. Extra copies of VirG and VirE can be particularly useful.

[0118] Additionally, it is convenient to include in the T-DNA a selectable marker that will allow identification and selection of transformed plant cells. The selectable marker should be one that works in both Agrobacterium and the target plant. For example, the genes encoding chloramphenicol acetyltransferase and neomycin phosphotransferase are suitable marker genes that confer resistance to chloramphenicol and kanamycin, respectively. Additionally, a selectable marker may be provided on a separate T-DNA from the T-DNA encoding the gene of interest. Co-transformed T-DNAs can integrate at separate sites in the plant genome. This can be useful because it will later allow segregation of the marker gene in progeny enabling the generation of transgenic trees expressing the gene of interest but not the marker gene.

[0119] The gene of interest and the selectable marker genes must also be under the control of promoters that function in the transformed plant cell. Examples of suitable promoters include, but are not limited to: the abscisic acid (ABA)-inducible promoter

LAW OFFICES
FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
202-408-4000